Elevated LDL cholesterol with a carbohydrate-restricted diet: evidence for a 'lean mass hyper-responder' phenotype

Norwitz et al.

Online Supplementary Material

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Supplemental Table 1. Low BMI is consistently associated with LDL cholesterol increase. Results of bootstrapped AIC-backward-stepwise analysis ranked in order of descending selection consistency. HDLc, HDL cholesterol; LDLc, LDL cholesterol; TG, triglycerides.

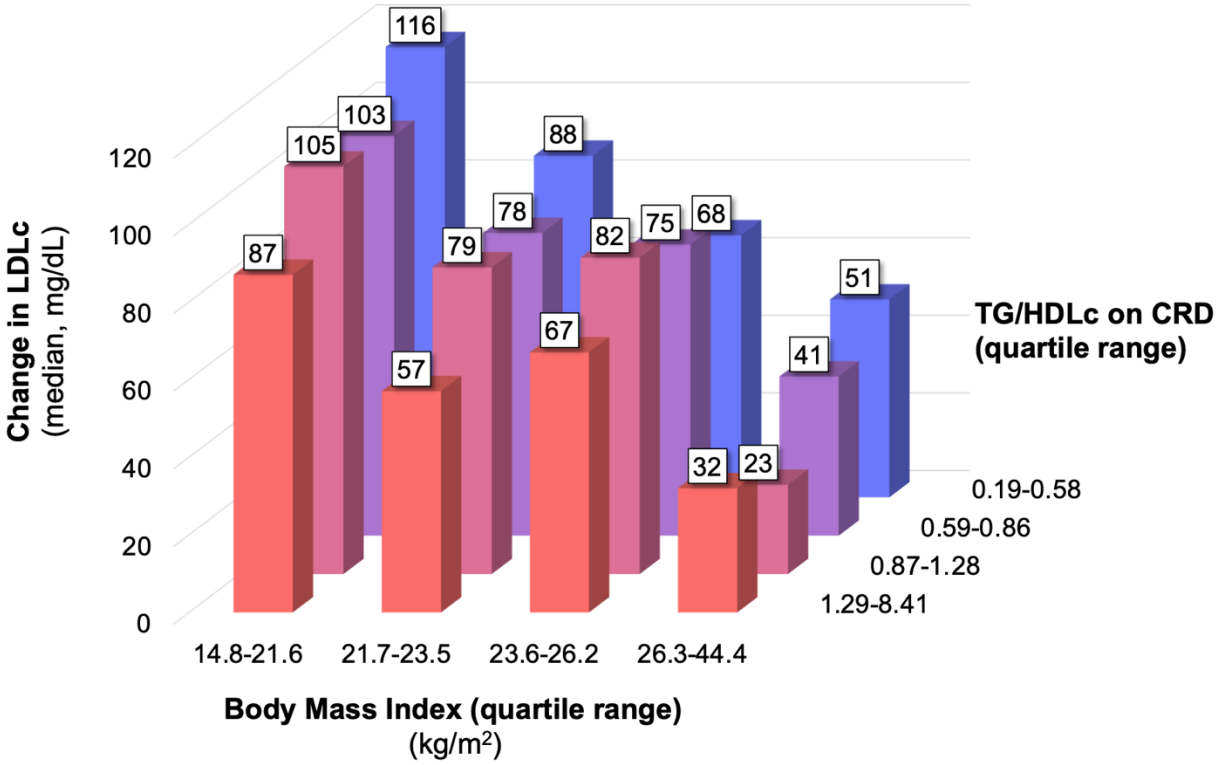
							Bootstrap AIC diagnostic analysis (B=100)		
	Model	***Term***	***β***	***SE***	***CI 95%***	***p-value***			
Δ LDLc	m0	Intercept	171	42.9	87.0 : 255.9	$7.5x10^{-5}$	Stat. Signif. Consistency	Coeff. Sign Consistency	AIC Selection Consistency
		BMI	-4.5	1.14	-6.71 : -2.22	$1.0\ x10^{-4}$	100%	100%	100%
		current HDLc	1.7	0.31	1.08 : 2.32	$8.2\ x\ 10^{-8}$	100%	100%	100%
		prior LDLc	-0.38	0.06	-0.51 : -0.25	$1.5\ x\ 10^{-8}$	100%	100%	100%
		current TG	0.34	0.28	-0.21 : 0.91	0.22	55%	100%	87%
		prior TG	-0.42	0.19	-0.79 : -0.04	0.03	80%	100%	91%
		Age	-0.72	0.34	-1.24 : -0.07	0.08	70%	100%	70%
		prior HDLc	-0.58	0.33	-0.8 : 0.41	0.53	79%	70%	65%
		prior TG/HDLc	-97.4	825	-1719 : 1524	0.90	62%	75%	67%
		current TG/HDLc	115	825	-1506 : 1738	0.88	55%	73%	54%
		Sex (male)	16.07	8.2	-10 : 32	0.05	79%	100%	65%

Supplemental Table 2. Prior TG/HDL cholesterol and BMI best predicts LDL cholesterol change on CRD. All-around performance of four models, depicted in Supplemental Figure 2, for explaining LDLc change on a CRD. HDLc, HDL cholesterol; LDLc, LDL cholesterol; TG, triglycerides. AIC: Akaike Information Criterion. BIC: Bayesian Information Criterion. R^2 adj.: Adjusted to the number of predictors in the model. RMSE: Root Mean Square Error. Sigma: Residual Standard Deviation of the errors.

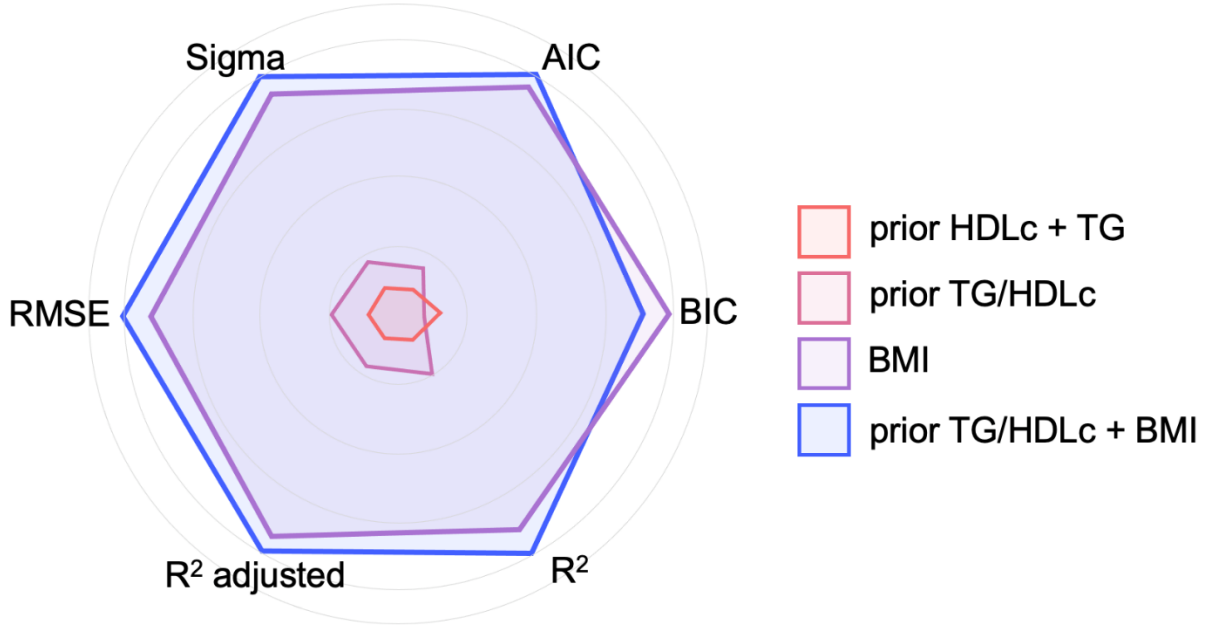
Outcome	*Model*	*AIC*	*BIC*	*R^2*	*R^2 adj.*	*RMSE*	*Sigma*
Δ *LDLc*	prior HDLc + prior TG	6618	6635	0.037	0.033	100.7	101.0
	prior TG/HDLc	6621	6635	0.027	0.025	101.2	101.4
	BMI	6597	6611	0.068	0.067	99.1	99.2
	prior TG/HDLc + BMI	6597	6614	0.073	0.07	98.8	99.1

Supplemental Table 3. Comparison of LMHR and non-LMHR subgroups in survey with US Nationally Representative data (NHANES IV). Both respondent subgroups had lower TG, higher HDL cholesterol, and higher LDL cholesterol, and lower BMI. HDLc, HDL cholesterol; LMHR, lean-mass hyper-responder; LDLc, LDL cholesterol; TG, triglycerides.

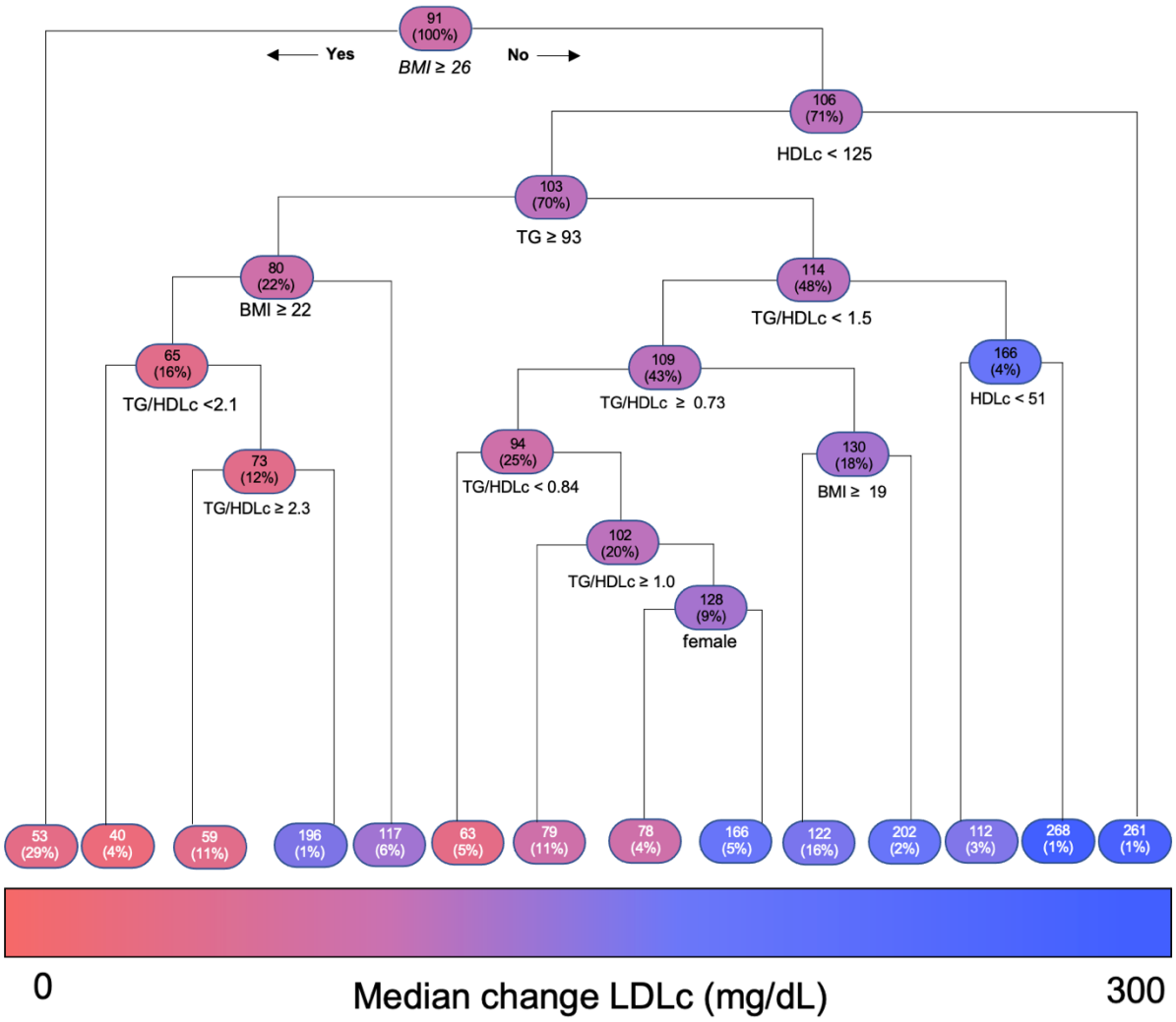
	LMHR			*non-LMHR*			*NHANES IV*		
	mean	SD	median	**mean**	SD	median	**mean**	SD	median
BMI (kg/m^2)	**22.0**	2.7	21.8	**24.6**	4.1	23.9	**28.5**	6.8	27.5
Current LDLc	**319.8**	114.8	286	**216.9**	96	191.0	**113.1**	35.2	111
Prior LDLc	**147.9**	64.5	132.5	**144.5**	58.0	134.5			
Current HDLc	**98.9**	16.3	95	**71.0**	20.0	70.0	**53.2**	15.0	51.0
Prior HDLc	**75.7**	22.1	72.0	**60.5**	19.0	58.0			
Current TG	**46.7**	14.6	46.0	**77.3**	38	72.0	**126**	94	102
Prior TG	**66.4**	56.8	57.0	**104.4**	68	84.5			



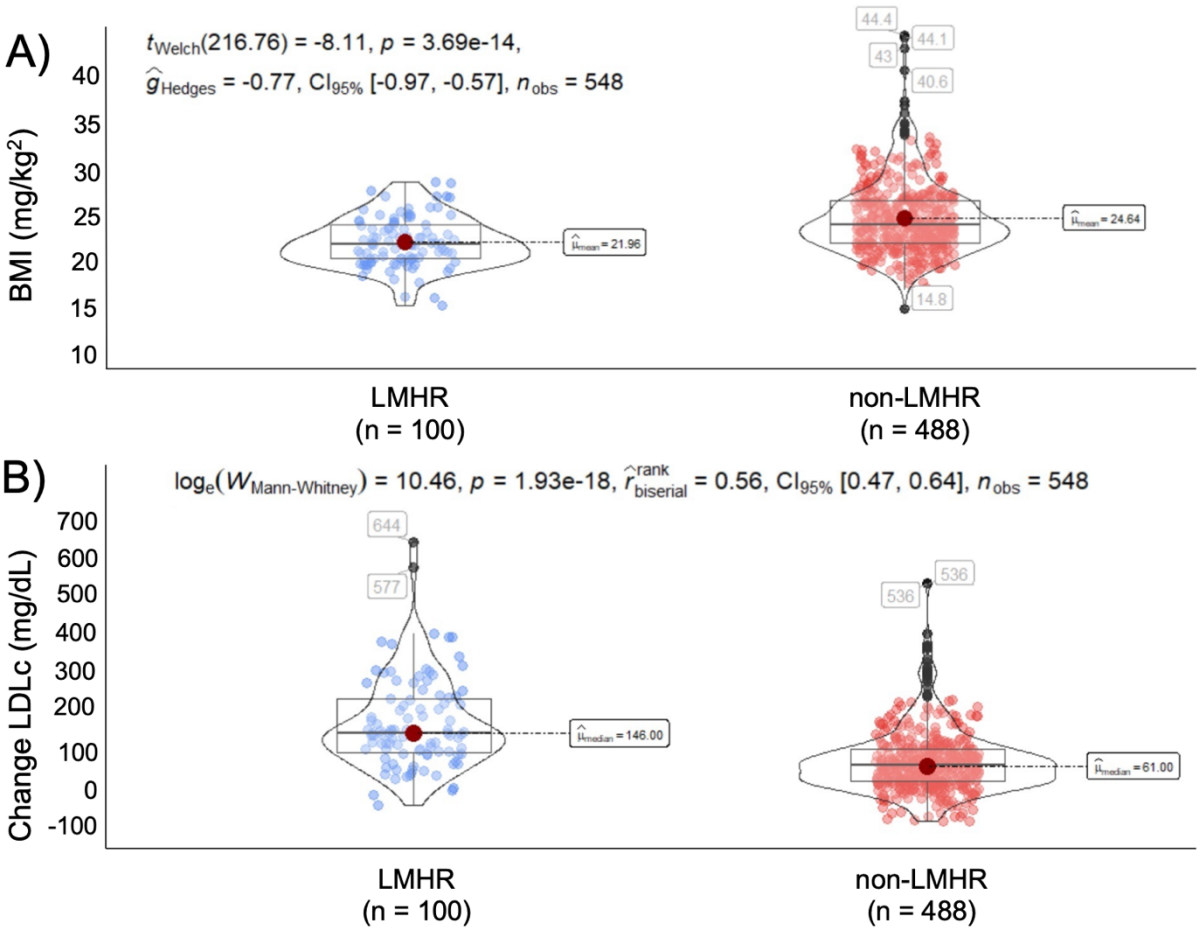
Supplemental Figure 1. BMI and current TG/HDL cholesterol are associated with LDL cholesterol increases on a CRD. Median LDL cholesterol change according to quartiles of TG/HDL cholesterol ratio on a CRD and of BMI (n = 34 per cell). HDLc, HDL cholesterol; LDLc, LDL cholesterol; TG, triglycerides.



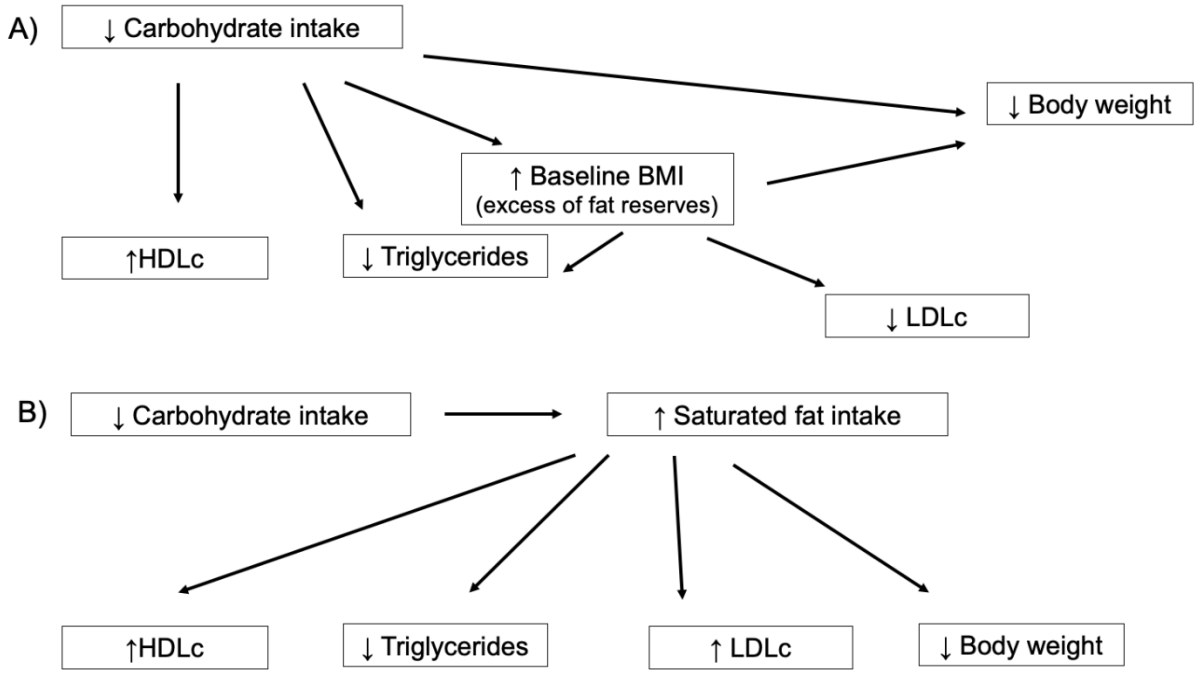
Supplemental Figure 2. Overall performance comparison of models explaining LDL cholesterol change on a CRD. The combination of prior TG/HDL cholesterol and BMI performs best (see Supplemental Table 2 for detail). HDLc, HDL cholesterol; LDLc, LDL cholesterol; TG, triglycerides. AIC: Akaike Information Criterion. BIC: Bayesian Information Criterion. R2 adj.: Adjusted to the number of predictors in the model. RMSE: Root Mean Square Error. Sigma: Residual Standard Deviation of the errors.



Supplemental Figure 3. Decision tree of LDL cholesterol change. BMI emerged as the first branch point (at 26 kg/m^2) in an independent and weight-threshold-naive fashion. Prior HDL cholesterol and TG were also identified as meaningful branchpoints. Percentages represent the proportion of participants discriminated at each node. This chooses the split that maximizes the between-groups sum-of-squares at each side of the node, however, is not enough to warrant such values are the optimal cut-off points. HDLc, HDL cholesterol; LDLc, LDL cholesterol; TG, triglycerides.



Supplemental Figure 4. Respondents with lipid-defined LMHR phenotype are leaner and exhibit larger LDL cholesterol increases than those without this phenotype. Violin-box plots with outlier tags and APA standard statistical assessment shown. Panel A shows mean BMI for LMHR and non-LMHR are 21.96 vs. 24.64 kg/m^2, p = 3.69 x 10^{-14}. Panel B shows mean LDL cholesterol change for LMHR and non-LMHR are 146 vs. 61 kg/m^2, p = 1.93 x 10^{-18}. HDLc, HDL cholesterol; LMHR, lean-mass hyper-responder; LDLc, LDL cholesterol; TG, triglycerides.



Supplemental Figure 5. Directed acyclic graphs illustrating possible causal relationships driving LDL cholesterol increases in the context of low BMI. Panel A) Causal relationships with BMI as modifier of the LDL changes effect of low-carbohydrate diets. Panel B: Saturated fat as a modifier factor of the effect of carbohydrate restriction on LDL cholesterol changes. Note that, if saturated fat is causal in determining LDL cholesterol increases, it must also associate with increases in HDL cholesterol and decreases in TG and BMI. HDLc, HDL cholesterol; LDLc, LDL cholesterol; TG, triglycerides.

Case Series

Patients all exhibited large LDL cholesterol increases on a very low-carbohydrate diet that were ameliorated by reintroduction of 50 – 100 grams of dietary carbohydrates.

Case 1 (I.A.)

I.A. is a 56-year-old man with a past medical history of hyperlipidemia, premature ventricular contractions, and degenerative disk disease. Family history was notable for paternal hypertension and type 2 diabetes. Genetic testing for familial hypercholesterolemia was negative. Medication included sildenafil. In 2019, BMI was 22.9 kg/m^2, and total body fat was 12.8%. Severe hyperlipidemia had developed over a three-year period after starting a very-low-carbohydrate diet (VLCD) in 2016. Coronary artery calcium score (CAC) in 2019 was 24 and carotid intima media thickness score (CIMT) was 0.07 cm on right and 0.09 cm on left.

Prior lipid data from 2015, before initiation of the VLCD, included total cholesterol 214 mg/dL, LDL cholesterol 116 mg/dL, HDL cholesterol 81 mg/dL, and TG 84 mg/dL. Laboratory data in 2019, after initiation of VLCD, showed severely elevated total cholesterol 797 mg/dL and LDL cholesterol 665 mg/dL, with HDL cholesterol 122 mg/dL, and TG 50 mg/dL. Additional testing in December 2019 (with LDL cholesterol 440 mg/dL) showed low levels of small LDL particles (<90 nmol/L; reference range <528 nmol/L), large LDL size (22.5 nm; Pattern A = 20.6 – 23.0 nm, B = 19.0 – 20.5 nm), low C-Reactive Protein (<0.2 mg/dL; ref <8.0 mg/dL), and normal fasting insulin leptin, TSH and testosterone. Lipoprotein insulin resistance (LPIR) score was optimal (<25) on seven occasions.

I.A. was counseled on standard-of-care pharmacotherapy and dietary changes for elevated LDLc, but declined to stop the VLCD or initiate lipid lowering pharmacotherapy. Six months after reintroduction of 50-100 grams/day carbohydrates in the form of starch or fruit, in

July 2020, total cholesterol decreased to 294 mg/dL and LDL cholesterol to 185 mg/dL, without a significant change in BMI.

Case 2 (M.I.)

M.I. is a 49-year-old woman with a past medical history of hyperlipidemia, severe osteoporosis, hypothalamic amenorrhea, anorexia nervosa, cardiomegaly, and vitamin deficiency. Family history was notable for type 2 diabetes, hypertension, hyperlipidemia, and hypothyroidism. Genetic testing for familial hypercholesterolemia was negative. Medications included vitamin D_3, zinc, multivitamin, trace mineral tablets, and Lugol's iodine solution. In 2019, BMI was 21.5 kg/m^2 and total body fat was 22.5%. CAC was 0 and CIMT was 0.05 cm bilaterally.

Lipid data from before initiation of the VLCD in 2018 included total cholesterol 209 mg/dL, LDL cholesterol 122 mg/dL, HDL cholesterol 72 mg/dL, and TG 54 mg/dL. Lipid analysis, after initiation of the VLCD in 2020, showed total cholesterol 698 mg/dL, LDL cholesterol 583 mg/dL, HDL cholesterol 97 mg/dL, and TG 70 mg/dL.

M.I. was counseled on standard-of-care pharmacotherapy and dietary changes but declined to stop the VLCD or initiate lipid lowering pharmacotherapy. After reintroduction of 50-100 grams of carbohydrates in the form of starch or fruit in August 2020, total cholesterol decreased to 497 mg/dL and LDL cholesterol to 360 mg/dL, without a significant change in BMI.

Case 3 (R.O.)

R.O. is a 44-year-old man with a past medical history of hyperactive thyroid nodules and hyperlipidemia. Family history was notable for obesity, hypertension, thrombosis, multiple

myeloma, myocardial infarction, and type 2 diabetes. Genetic testing for familial hypercholesterolemia was negative. Medications included magnesium, omega-3 fish oil, vitamin C, and multivitamin. In 2020, BMI was 30.1 kg/m^2 and, total body fat was 24.8%. CAC was 4.

Lipid data from before initiation of the VLCD in February 2020 showed total cholesterol 197 mg/dL, LDL cholesterol 137 mg/dL, HDL cholesterol 45 mg/dL, and TG 62 mg/dL. Lipid data in October 2020, after initiation of the VLCD, showed total cholesterol 311 mg/dL, LDL cholesterol 239 mg/dL, HDL cholesterol 65 mg/dL, and TG 56 mg/dL.

R.O. was counseled on standard-of-care pharmacotherapy and dietary changes but declined to stop the VLCD or initiate lipid lowering pharmacotherapy. After reintroduction of 50 to 100 grams/day carbohydrate, lipid in March 2021 showed total cholesterol decreased to 180 mg/dL and LDL cholesterol to 115 mg/dL without a significant change in BMI.

Case 4 (N.M.)

N.M. is a 56-year-old man with a past medical history of atrial fibrillation, testicular cancer, gastroesophageal reflux disease with Barret's esophagus, autoimmune thyroiditis, and hyperlipidemia. Family history was notable for coronary artery disease, and thyroid and prostate cancer. Genetic testing for familial hypercholesterolemia was negative. Medications included aspirin, verapamil, pantoprazole, omega-3-acid ethyl esters, and CoQ_{10}. In 2019, BMI was 27.1 kg/m^2 and total body fat of 21%. CAC was 1 and CIMT was 0.05 cm bilaterally.

Lipid data from before initiation of the VLCD in 2016 showed total cholesterol 179 mg/dL, LDL cholesterol 113 mg/dL, HDL cholesterol 49 mg/dL, and TG 86 mg/dL. After starting a VLCD in August 2019, total cholesterol increased to 387 mg/dL and LDL cholesterol increased to 317 mg/dL, with HDL cholesterol 59 mg/dL, and TG 54 mg/dL.

N.M. had prior intolerance to statins. After reintroduction of 50 to 100 grams/day carbohydrate in the form of starch or fruit, lipid testing in August 2020 showed total cholesterol decreased to 272 mg/dL and LDL cholesterol to 195 mg/dL without a significant change in BMI.

Case 5 (A.N.)

A.N. is a 58-year-old man with a past medical history of pulmonary embolism and hyperlipidemia. Family history was notable for a maternal history of hypertension and paternal history of chronic obstructive pulmonary disease. Genetic testing for familial hypercholesterolemia was negative. Medications included apixaban. In 2019, BMI was 27.8 kg/m^2 and total body fat was 22.3%. CAC was 33.2 and CIMT was 0.06 cm bilaterally.

In 2014, lipid data prior to beginning the VLCD showed total cholesterol 218 mg/dL, LDL cholesterol 141 mg/dL, HDL cholesterol 57 mg/dL, and TG 98 mg/dL. A.N. lost 80 pounds after twelve months on a VLCD. In October 2019, after weight loss, lipids data showed total cholesterol 423 mg/dL, LDL cholesterol 336 mg/dL, HDL cholesterol 69 mg/dL, and TG 74 mg/dL. Due to the successful weight loss on the VLCD on weight loss, A.N. declined standard dietary treatment for elevated LDL cholesterol and also declined pharmacotherapy. After reintroduction 50-100 grams/day carbohydrate in the form of starch or fruit in June 2020, lipid testing showed total cholesterol decreased to 318 mg/dL and LDL cholesterol to 236 mg/dL without a significant change BMI.

Survey

Full “Cholesterol Super Survey.” Only questions on height, weight, age, sex, carbohydrate intake, and current and prior lipids were utilized for the purposes of this manuscript.

IMPORTANT (1 of 2): Please fill this out carefully and HONESTLY. It's important we gather accurate data. Thank you!

IMPORTANT (2 of 2): You may or may not be contacted after submission. Please watch for updates at this dedicated page: https://www.cholesterolcode.com/supersurvey

First Name

[Short answer text]

Last Name

[Short answer text]

Date of Birth

[Month, day, year]

NOTE: Personal information (Name, date of birth) will be only for INTERNAL USE by the Cholesterol Code team and will not be released publicly.

Age

[Short answer text]

Height (examples: 6'2 or 188cm)

[Short answer text]

Weight (examples: 170lb or 77kg)

[Short answer text]

Gender

[Male/Female/Prefer not to say]

City

[Short answer text]

State

[Short answer text]

What is a ballpark of your average of your daily calories?

[Short answer text]

About how many grams of protein do you typically consume in a day?

[Short answer text]

About how many grams of fat do you typically consume in a day?

[Short answer text]

About how many grams of net carbs do you consume in a day? (Net carbs = total carbs - fiber)

[Short answer text]

List any supplements you take such as vitamins, fish oil, etc.

[Long answer text]

List any medications you are taking

[Long answer text]

In a typical week, about how many hours do you spend doing cardiovascular / aerobic exercise?

[Short answer text]

In a typical week, about how many hours do you spend doing HIIT (high intensity interval training) / anaerobic exercise?
[Short answer text]

In a typical week, about how many hours do you spend doing weight lifting / resistance training?
[Short answer text]

Is there a history of early heart disease or stroke in your family?
[Yes / No]

If you've had a Coronary Artery Calcium (CAC) test before, what was the most recent score? (Leave blank if you've never had one.)
[Short answer text]

Have you had your genetics tested with a service such as 23andMe?
[Yes / No]

Most Recent Bloodwork (on this current diet)
Please fill out the details on your MOST RECENT cholesterol blood test

When was this test taken?
[Month, day, year]

Were you water-only fasted for 12-14 hours before the cholesterol test? If not, write in how long.
[Yes]
[Other] [Short answer text]

In what units are your blood measurements? *(usually mg/dL in the US, mmol/L outside the US)*
[mg/dL] *(Usually numbers in the tens to hundreds - example: 112)*
[mmol/L] *(Usually numbers under 20 in the decimals - example: 4.3)*

Total Cholesterol:
[Short answer text]

LDL Cholesterol (LDL-C):
[Short answer text]

HDL Cholesterol (HDL-C):
[Short answer text]

Triglycerides (TG):
[Short answer text]

Last bloodwork taken BEFORE beginning your current diet
Please fill out the details on the last cholesterol blood test you took BEFORE beginning the diet you're on now

When was this test taken?
[Month, day, year]

Were you water-only fasted for 12-14 hours before the cholesterol test? If not, write in how long.
Yes
Other [Short answer text]

In what units are your blood measurements? (usually mg/dL in the US, mmol/L outside the US)
[mg/dL] *(Usually numbers in the tens to hundreds - example: 112)*
[mmol/L] *(Usually numbers under 20 in the decimals - example: 4.3)*

Total Cholesterol:
[Short answer text]

LDL Cholesterol (LDL-C):

[Short answer text]

HDL Cholesterol (HDL-C):
[Short answer text]

Triglycerides (TG):
[Short answer text]

Do you have any interest of participating in studies on a wide variety of topics? And if so, may we contact you through the email you provided above?
[Yes / No]